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Inventors	: Hiroo Kumagai
	: Takao Saruta
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	: EXAMINING
	: DISEASE

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#11
M.Q.
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Declaration of Mr. Kenji Takamori

Commissioner for Patents
Washington, DC 20231

Sir:

I, Kenji Takamori, declare:

I am a citizen of Japan, residing at 5-24 Momijigaoka 1-chome, Fuchu-shi, Tokyo, Japan; I am the chairman of and a professor in the Department of Dermatology, Juntendo University Urayasu Hospital; I graduated from Juntendo University School of Medicine in 1968 and Graduate School of Medicine in 1972; I have had appointments as a research associate of Duke University in 1977, assistant professor of Department of Dermatology, Juntendo University in 1987 and professor of Department of Dermatology, Juntendo University in 1993; I have been the chairman of and a professor in the Department of Dermatology, Juntendo University Urayasu Hospital from 2002;

I am a member of the following scientific societies:

Japanese Society for Investigative Dermatology (Administration Officer),
Japanese Dermatological Association (Councilor),
Japanese Society for Psoriasis Research (Councilor),
Journal of Dermatological Science (Associate Editor, 1990 - 1992);

I have published scientific papers in Attachment 1;

I have been engaged in research of biochemistry of dermatology, pathophysiology of pruritus and treatment of skin diseases including atopic dermatitis for may years. I am familiar with the above-identified application.

The experiments set forth below were conducted under my direct guidance and supervision, and the results presented in the experiments have been carefully checked and are indeed truly correct.

Diagnosis and treatment of pruritus by opioids

(1) Diagnosis of itching. by the measurement of serum opioid peptides

Serum concentrations of two types of opioid peptides (beta-endorphin and dynorphin A) in healthy subjects and atopic dermatitis patients were measured as described in Example 2 of the Applicants' specification.

Averaged serum concentration of beta-endorphin (opioid mu agonist) ware 28 pg/mL and 87.7 pg/mL in healthy subjects (n=12) without itching and atopic dermatitis patients (n=10) with severe. itching, respectively. The ratio of beta-endorphin by dynorphin A (opioid kappa agonist) were 2.2 for healthy subjects and 3.3 for atopic dermatitis patients. This showed that the ratio (beta-endorphin/dynorphin A) was increased as corresponded to the severe itching.

(2) Opioid receptor. expression on human. peripheral lymphocytes

The level of opioid receptor expression (mu and kappa) on human peripheral lymphocytes was determined as described in the above-mentioned Example 2.

The balance of the level of opioid receptor expression is shown in Table 4. Kappa receptors were dominantly expressed in healthy subjects as compared with mu receptors, but relatively expressed more than kappa receptors in atopic dermatitis patients with severe itching. This suggests that mu receptor was relatively expressed more than the kappa receptor in the itchy state.

Table 4. Comparison of the expression of mu and kappa receptors on human peripheral lymphocytes

Subject	mu>kappa	mu=kappa	mu<kappa	N
Healthy	21%	44%	35%	43
Atopic dermatitis	49%	51%	0%	41

(3) Itch treatment by opioid kappa agonist

Three atopic dermatitis patients who suffer from severe itching resistant to conventional anti-histamine drugs were enrolled for the evaluation of opioid kappa agonists. One hundred to two hundred micrograms of the vaseline-based ointment. containing an opioid kappa agonist, (-)-17-(cyclopropylmethyl)-3,14 beta-dihydroxy-4,5 a-epoxy-6 beta-[N-methyl-trans-3-(3-furyl) acrylamido] morphinan hydrochloride (10 mcg/g) as an ingredient, and vehicle were independently applied to itching sites of each patient under blind control. Itching sensation disappeared in 15 minutes after topical treatment at all sites of three patients. This showed that the opioid kappa agonist expressed an antipruritic effect.

Thus, the endogenous opioid system is influential to itching in atopic dermatitis patients. This factually supports the method of evaluation by the ratio of beta-endorphin/dynorphin A and the treatment by opioid kappa agonist.

It is declared by the undersigned declarant that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001, of Title 18, of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

March 24, 2003
Date

Kenji Takamori

Attachment 1

1. Palungwachira P, Kakuta M, Yamazaki M, Yaguchi H, Tsuboi R, Takamori K, Ogawa H.
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2. Palungwachira P, Chirachanakul P,. Palungwachira P, Chalugan P, Nakao A, Takamori K, Ogawa M.
Cutaneous findings in HIV-1-positive patients in Thailand. J Dermatol. 2001 Oct; 28(10):584-5. No abstract available.

3. Jindo T, Tsuboi R, Takamori K, Ogawa H.
Local injection of hepatocyte growth factor/scatter factor (HGF/SF) alters cyclic growth of murine hair follicles. J Invest Dermatol. 1998 Apr; 110(4):338-42.

4. Yamada H, Suga Y, Takamori K, Ogawa H.
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7. Takamori K, Yoshiike T, Morioka S, Ogawa H.
The role of proteases in the pathogenesis of bullous dermatoses. Int J Dermatol. 1988 Oct; 27(8):533-9.

8. Takamori K, Ikeda S, Naito K, Ogawa H.
Proteases are responsible for blister formation in recessive dystrophic epidermolysis bullosa and epidermolysis bullosa simplex. Br J Dermatol. 1995 May; 112(5):533-8.

9. Takamori K, Naito K, Ogawa H.
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10. Takamori K, Naito K, Taneda A, Ogawa H.
Increased neutral protease and collagenase activity in recessive dystrophic epidermolysis bullosa. Br J Dermatol. 1983 Jun; 108(6):687-94.